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Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of N-methylcarbamate Pesticides in Surface Water using High Performance Liquid Chromatography and Post-column derivatization

1. Scope:

   This section method (SM) documents the selected N-methylcarbamate pesticides analysis in surface water by all authorized section personnel.

2. Principle:

   The surface water sample is extracted with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated to almost dryness then diluted to a final volume of 0.40 mL with methanol. The extract is then analyzed by HPLC. The analytes are derivatized with OPA (ortho-phthaladehyde) in a post column reaction and detected with a fluorescence detector. The reporting limit for this method is 0.05 ppb for all compounds.

3. Safety:

   3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.

   3.2 Methylene chloride is a regulated and controlled carcinogenic hazardous substance. It must be stored and handled in accordance with California Code of Regulations, Title 8, Subchapter 7, Group 16, Article 110, Section 5202.

   3.3 All solvents should be handled with care in a ventilated area.

4. Interferences:

   There are matrix interferences that cause quantitative problems. Therefore the calibration standards will be made up in appropriate matrix.

5. Apparatus and Equipment:

   5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
   5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
   5.3 Vortex-vibrating mixer
   5.4 Balance (Mettler PC 4400) or equivalent

5.5 HPLC with post column derivatization system and fluorescence detector.

6. Reagents and Supplies

6.3 Methylene Chloride, nanograde or equivalent pesticide grade
6.4 Methanol, nanograde or equivalent pesticide grade
6.5 Anhydrous Sodium Sulfate, granular
6.6 Aldicarb Sulfoxide CAS# 1646-87-3
6.7 Aldicarb Sulfone CAS# 1646-88-4
6.8 Oxamyl CAS# 23135-22-0
6.9 Methomyl CAS# 16752-775
6.10 3-OH-Carbofuran CAS# 16655-82-6
6.11 Aldicarb CAS# 116-06-3
6.12 Carbofuran CAS# 1563-66-2
6.13 Carbaryl CAS# 63-25-2
6.14 Methiocarb CAS# 2032-65-7
6.15 Hydrolysis reagent (Pickering Laboratories CB130 or equivalent)
6.16 O-phthaladehyde (Pickering Laboratories 012 or equivalent)
6.17 O-phthaladehyde diluent (Pickering Laboratories CB910 or equivalent)
6.18 2-mercaptoethanol
6.19 OPA Reagent- Dissolve 100mg O-Phthaladehyde in 10mL methanol. Add this mixture to 950 mL O-Phthaladehyde diluent and mix well. Add 1 mL 2-mercaptoethanol and pour solution into reagent reservoir.
6.20 Conical tube with glass stopper, 15-mL graduated, 0.1 mL subdivision
6.21 Separatory funnel, 250 mL
6.22 Boiling flask, 500 mL
6.23 Funnel, long stem, 10 mm diameter
6.24 Nitrogen Evaporator, Organomation
6.25 Disposable Pasteur pipettes, and other laboratory ware as needed
6.26 0.2µ nylon filters (Acrodisc 28143-274 or equivalent)
6.27 Recommended analytical columns:
Carbamate analysis C18 4.6mm ID X 250 mm. (Pickering Laboratories 1846250 or equivalent)

7. Standards Preparation:

7.1 Dilute the 1 mg/mL Carbamate standards obtained from the CDFA/CAC Environmental Analysis Standards Repository with methanol to make up a series of

mixed working standards (see 10.2). These standards shall be prepared to cover the linear range from 0.0125 ηg/μL to 0.5 ηg/μL for the carbamate screen.

7.2 Store standards according to manufacturing requirement. Keep all standards in designated refrigerator for storage.

7.3 The expiration date of each mixed working standard is six months from the preparation date or same as stock standards, if sooner.

8. Sample Preservation and Storage:

All water samples and sample extracts shall be stored in the refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator and allow samples to come to room temperature before extraction.

9.1.2 Preparation of matrix blank and matrix spike:

The Department of Pesticide Regulations (DPR) provides the background water for matrix blank and spikes.

9.1.2.1 Matrix blank: Weigh out 100 grams of background water and follow the test sample extraction procedure.

9.1.2.2 Matrix spike: Weigh out 100 grams of background water. Spike a client requested amount of carbamate pesticides into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

9.2 Test Sample Extraction

9.2.1 Shake each sample then weigh out 100 grams of sample and transfer to a separatory funnel.

9.2.2 Shake with 100 ± 5 mL of methylene chloride for 2 minutes. Vent frequently to relieve pressure.

9.2.3 After phases have separated, drain lower methylene chloride layer through 20 ± 4 g of anhydrous sodium sulfate and glasswool, into a 500 mL boiling flask.

9.2.4 Repeat steps 9.2.2 & 9.2.3 two more times using 100 ± 5 mL of methylene chloride each time. Combine the extracts in the same boiling flask.

9.2.5 After draining the final extraction, rinse the sodium sulfate with 25 ± 5 mL of methylene chloride.

9.2.6 Evaporate the sample extract to 2 - 4 mL on a rotary evaporator using a water bath at 35 ± 2 °C and 15 - 20 inch Hg vacuum. Pass sample through 0.2µ filter into a calibrated 15 mL graduated test tube.

9.2.7 Rinse flask 2-3 more times with 2 - 4 mL of methylene chloride and filter the rinse into the same test tube.

9.2.8 Evaporate the extract to a volume slightly less than 0.5 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Add in approx. 1 mL methanol. Evaporate the extract to less than 300 µL. Transfer extract to a calibrated vial insert. Wash the tube with a few drops of Methanol and add to insert. Adjust the final volume of 0.4 mL with methanol.

9.2.9 Submit extract for HPLC analysis.

10. Instrument Calibration:

10.1 A calibration standard curve consists of minimum of three levels. Standard concentrations of 0.0125, 0.025, 0.05, 0.1, and 0.5 ηg/µL are recommended. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995.

10.2 Compositions of calibration mixed standards are as follows:

CB-A Mixed Standard
Aldicarb Sulfoxide
Aldicarb Sulfone
Methomyl
3-Hydroxycarbofuran
Aldicarb
Carbofuran
Carbaryl

CB-B Mixed Standard
Oxamyl
Methiocarb

11. Analysis:

11.1 Injection Scheme

Follow the sequence of calibration standards, QC samples, test samples (maximum of 10-12 samples) and final calibration standards.

11.2 HPLC Instrumentation

11.2.1 Analyze carbamate pesticides by HPLC equipped with post column reaction module and a fluorescence detector.

11.2.2 Recommended instrument HPLC gradient::

A= 1% methanol in water B= acetonitrile

Time (min)	% A	%B
0.00	98.0	2.0
1.00	98.0	2.0
16.00	30.0	70.0
18.00	30.0	70.0
22.00	100.0	0.0
25.00	100.0	0.0
25.10	98.0	2.0
30.00	98.0	2.0

11.2.3 Injection volume 25 µL.

12. Quality Control:

12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample.

12.2 The matrix blank should be free of target compounds.

12.3 The recoveries of the matrix spike shall be within the control limits.

12.3.1 When spike recoveries fall outside the control limits, the chemist must investigate the cause. The entire extraction set of samples is re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples shall be reported.

12.3.2 If the spike recoveries still fall outside the control limits, the client will be notified. The backup samples will be re-extracted for analysis.

12.4 The retention time should be within $\pm$ 2 percent of that of the standard.

12.5 The sample must be diluted if results fall outside the linear range of the standard curve.

12.6 Bracketing standard curves should have a percent change less than 20 % for all compounds.

12.7 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analytel that a method can detect reliably. To determine the MDL, 7 replicate water samples are spiked at 0.05 ppb for OP screen and 7 replicate water samples are spiked at 10 ppt for low level diazinon and chloripyrifos. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the follow equation:

$$MDL = tS$$

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicate used to determine the MDL, t=3.143.

12.8 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Per client agreement, the RL is chosen in a range 1-5 times the MDL except in special cases. (See 15.5)

MDL data and the RL are tabulated in Appendix IA and IB.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of three sample sets. Each set included five levels of fortification (0.0125, 0.025, 0.05, 0.1, 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method.

12.9.2 Upper and lower warning and control limits are set at $\pm$ 2 and $\pm$ 3 standard deviations of the average % recovery, respectively.

12.10 Estimated Measurement Uncertainty:

12.11 Trend Identification

12.11.1 All matrix spike recoveries for carbamate analysis will be put into control

Charts and monitored for trends. Three trend characteristics will be evaluated at least bi-yearly by the supervisor or designee.
2 of 3 points above or below 2/3 of the UCL or LCL.
7 continuous points above or below the center line (CL)
14 points alternating above and below the CL.

12.11.2 When results indicate an out of control situation the supervisor or designee will indicate this on the control chart and take appropriate corrective action, which may include monitoring the results more closely to initiating a formal corrective action with root cause investigation.

13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The software uses a linear or quadratic curve fit, with all levels weighted equally. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$ppb = \frac{(\text{sample peak ht. or area})\ (\text{std. conc.})\ (\text{std. vol. injected})\ (\text{sample final vol., (mL)})(1000\ \mu\text{L/mL})}{(\text{std. peak ht. or area})\ (\text{sample vol. injected})\ (\text{sample wt., g})}$$

14. Reporting Procedure:

14.1 Identification of Analyte

For responses within calibration range, compare the retention time of the peaks with the retention time of standards. For positive results retention times shall not vary from the standards more than 2 percent.

14.2 Sample results are reported out according to the client's analytical laboratory specifications.

15. References:

Muth, G.L., Erro, F. A Rapid Carbamate Multiresidue Procedure of Vegetable Crops Environmental Contamination & Toxicology, 1980, 24, 759-765

Keith, Lawrence H., Principles of Environmental Analysis, Anal Chem, 1983, 55, 2210-2218

APPENDIX IA

The determination of Method Detection Limit (MDL) data and Reporting Limit (RL)

	Aldicarb sulfoxide	Aldicarb sulfone/Oxamyl	Methomyl	3-OH Carbofuran	Aldicarb	Carbofuran	Carbaryl	Methiocarb
MDL#1	0.02433	0.06784	0.02939	0.0322165	0.024847	0.02892	0.03208	0.03113
MDL#2	0.02126	0.05778	0.02545	0.02704	0.02244	0.0267	0.02718	0.02444
MDL#3	0.0248	0.06608	0.02709	0.03169	0.02316	0.02691	0.02924	0.02875
MDL#4	0.02172	0.05155	0.02367	0.02685	0.02164	0.02417	0.0245	0.03718
MDL#5	0.01686	0.05218	0.02204	0.02594	0.01776	0.02235	0.02388	0.02301
MDL#6	0.02388	0.05906	0.029	0.03161	0.02579	0.02815	0.02841	0.02617
MDL#7	0.026	0.06423	0.03	0.035	0.0245	0.03114	0.03286	0.02866
SD	0.00307	0.00651	0.00306	0.00343	0.00268	0.00294	0.00344	0.00473
3.1416 xSD	0.01026	0.01882	0.00967	0.01133	0.00871	0.00964	0.01038	0.01578
MDL	0.011	0.020	0.010	0.011	0.010	0.010	0.011	0.016

All concentrations are expressed in ppb.

APPENDIX IIA

Method Validation Data and Control Limit for Carbamates Table 1

Level **µg/L** (ppb)	Aldicarb Sulfoxide	Percent recovery	Aldicarb Sulfone	Percent recovery	Methomyl	Percent recovery	3-OH-Carbofuran	Percent recovery	Aldicarb	Percent recovery
0.0125	0.0062	49.6	0.0071	56.8	0.0070	56.0	0.0101	80.8	0.0085	68.0
	0.0128	102.4	0.0178	142.4	0.0140	112.0	0.0131	104.8	0.0140	112.0
	0.0091	72.8	0.0108	86.4	0.0114	91.2	0.0138	110.4	0.0099	79.2
0.025	0.0261	104.4	0.0206	82.4	0.0201	80.4	0.0225	90.0	0.0185	74.0
	0.0203	81.2	0.0371	148.4	0.0268	107.2	0.0199	79.6	0.0181	72.4
	0.0220	88.0	0.0237	94.8	0.0213	85.2	0.0187	74.8	0.0224	89.6
0.05	0.0518	103.6	0.0439	87.8	0.0438	87.6	0.0448	89.6	0.0358	71.6
	0.0499	99.8	0.0426	85.2	0.0404	80.8	0.0442	88.4	0.0395	79.0
	0.0330	66.0	0.0491	98.2	0.0440	88.0	0.0433	86.6	0.0372	74.4
0.10	0.0838	83.8	0.0847	84.7	0.0818	81.8	0.0852	85.2	0.0706	70.6
	0.0832	83.2	0.0893	89.3	0.0764	76.4	0.0845	84.5	0.0722	72.2
	0.0852	85.2	0.0866	86.6	0.0853	85.3	0.0914	91.4	0.0742	74.2
1.00	0.7551	75.5	0.8672	86.7	0.8135	81.4	0.8269	82.7	0.7351	73.5
	0.7560	75.6	0.9580	95.8	0.8930	89.3	0.8920	89.2	0.8320	83.2
	0.7126	71.3	0.8458	84.6	0.7824	78.2	0.7595	76.0	0.6856	68.6

Method Validation Data and Control Limit for Carbamates Table 2

Level **µg/L** (ppb)	Carbaryl	Percent recovery	Oxamyl	Percent recovery	Methiocarb	Percent recovery
0.0125	0.0156	124.8	0.0094	75.2	0.0108	86.4
	0.0178	142.3	0.0108	86.4	0.0119	95.2
	0.0145	116.0	0.0104	83.2	0.0123	98.4
0.025	0.0292	116.8	0.0233	93.2	0.0236	94.4
	0.0248	99.2	0.0236	94.4	0.0333	133.2
	0.0391	156.4	0.0222	88.8	0.0216	86.4
0.05	0.0468	93.6	0.0420	84.0	0.0563	112.6
	0.0468	93.6	0.0565	113.0	0.0633	126.6
	0.0610	122.0	0.0514	102.8	0.0493	98.6
0.10	0.0897	89.7	0.0926	92.6	0.1066	106.6
	0.0897	89.7	0.0990	99.0	0.1040	104.0
	0.0989	98.9	0.0892	89.2	0.0956	95.6
1.00	0.8674	86.7	0.9215	92.2	0.8901	89.0
	0.9390	93.9	0.9930	99.3	0.9920	99.2
	0.8226	82.3	0.9027	90.3	0.9012	90.1

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Revision Log:

Date	What was Revised? Why?
3/11/09	Changed method validation results to the validation results done in June 2007.